Quantitative PCR Protocol for First Strand cDNA Synthesis with SuperScript™ III: Invitrogen® First Strand Synthesis System

This protocol is for use with the Invitrogen SuperScript III First strand synthesis system. For additional technical services please contact Invitrogen and www.invitrogen.com.

BEFORE STARTING THE EXPERIMENT

1. The RNA must be stored at -80° C at a concentration of 1µg in 5µl of DEPC water
2. Take RNA out of freezer to thaw. RNA should be thawed in a clean room and on ice; this is to prevent contamination and degradation of the RNA.
3. Thaw the reagents being used for the experiment in the clean room. SuperScript™ III and RNaseOUT™ should be thawed on ice.

SuperScript™III Protocol

Step A. Cocktail- for a final reaction volume of 50µl, to be added in this order: Added to each well being used for RT PCR

1. 10x RT buffer-	***5µl***
2. 50µM Oligo (dt) 20-	***2.5µl***
3. 10mM dNTP mix-	***2.5µl***
4. 25mM $MgCl_2$-	***10µl***
5. 0.1 M DTT-	***5µl***
6. DEPC Water-	***15µl***
7. RNaseOUT™ (40 U/µl)	***2.5µl***
8. SuperScript™III (200 U/µl)	***2.5µl***

Step B. Add 5µl of RNA to each well with the cocktail mix. Mix reagents and spin down briefly.

Step C. Incubation conditions:

Incubate the reaction for 50min at 50°C, inactivate the reaction for 5min at 85°C, and let cool to 4°C. Then place on ice.

Step D. To each well add 1µl of E.coli RNase H (2 U/µl)

Step E. Incubation conditions:

Incubate the reaction for 20min at 37°C and let cool to 4°C.

Step F. Storage conditions:

Use cDNA immediately for quantitative PCR or store at -20°C for future use.
